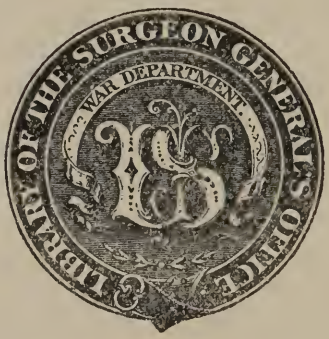
Water Analysis

CLARK



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WATER ANALYSIS

FOR

SANITARY PURPOSES

CHEMICAL AND BIOLOGICAL

BY

CHARLES H. CLARK, A. M.

PRINCIPAL OF SANBORN SEMINARY

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PREFACE.

The following pages, with the exception of the last two chapters, were originally compiled for use in the writer's classes, making as he believes a fitting conclusion to a year's work in General Chemistry and Qualitative Analysis by introducing the student to some of the processes of Quantitative Analysis. The work is given in the simplest form and does not even presuppose a knowledge of Qualitative Analysis. It has been successfully performed by a number of different classes and has been found not too difficult for the average student. Interest has always been aroused.

In giving these pages to the public the writer hopes to serve his fellow teachers by saving them a large measure of the labor it has cost him to put the subject of water analysis in a form to be presented to young students.

He trusts also that physicians may find these pages useful. The busy physician feels the lack of suitable simple processes for forming accurate judgments on the potableness of water. The writer would suggest that if time does not permit a complete examination, the analysis for albuminoid ammonia and chlorine and the testing for poisonous metals may be very quickly accomplished, and that much dependence may be placed in the results. The apparatus is not at all expensive and the solu-

tions may be compounded at the drug store. It is also hoped that physicians may be interested in the simple processes for the microscopical and biological examination.

Nearly all the apparatus and chemicals called for in the chemical analysis will be found even in a small laboratory. Processes calling for costly apparatus and unusual chemicals have been excluded. The balance and platinum evaporating dish are the only expensive pieces used. A porcelain evaporating dish may be substituted for the platinum; the results will be less accurate, but with careful work the errors will not be large enough to preclude the drawing of correct conclusions. A distilling apparatus of the form known as Liebig's Condenser may be purchased complete or easily improvised from glass tubing. It is desirable to have a burette for each standard solution, but one burette may be used for all the work. It should be carefully washed after use and left full of water. It has not seemed necessary to describe the method of using it, as such descriptions may be found in almost any treatise on chemistry.

Indebtedness is acknowledged to existing works on this subject for the facts and processes described. Material has been gathered from all available sources. An effort has been made to be concise in statement and clear in description with the aim of awakening an interest in this important subject.

C. H. C.

KINGSTON, N. H., July 29, 1892.

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CONVENIENT DATA.

Parts per 100,000 × .7 = grains per imperial gallon.

Grains per imperial gallon ÷ .7 = parts per 100,000.

Parts per 100,000 × .583 = grains per U. S. gallon.

Grains per U. S. gallon ÷ .583 = parts per 100,000.

Grains per imperial gallon × .8339 = grains per U. S. gallon.

Parts per 100,000 × .01 = grams per liter.

Grains per imperial gallon ÷ 70 = grams per liter.

1 liter = .264 U. S. gallon = .22 imperial gallon.

1 U. S. gallon = .8339 imperial gallon = 3.785 liters = 58,372 grains.

1 imperial gallon = 1.2 U. S. gal. = 4.543 liters = 70,000 grains.

1 cubic foot = 7.48 U. S. gallons = 6.232 imperial gallons = 28.315 liters.

$\frac{5}{9}$ (degrees Fahrenheit — 32°) = degrees Centigrade.

$\frac{9}{5}$ degrees Centigrade + 32° = degrees Fahrenheit.

WATER ANALYSIS.

CHAPTER I.

IMPORTANCE OF WATER ANALYSIS.

That pure water is important to health does not admit of question. There may be a difference of opinion as to the influence of the impurities in water in causing particular diseases, but it is an undisputed fact that certain diseases have followed the use of polluted water. Pure water, when examined under the microscope, is perfectly transparent. If water containing organic matter in solution be exposed to the air for some time, it will become turbid. The microscope shows that this turbidity is caused by minute living organisms of various shapes. Many are rod-shaped, and all are called *bacteria* from a Greek word meaning *rod*. The investigations of science have conclusively shown that bacteria cause disease. In certain diseases, forms of bacteria are found in the

human system identical with those found in impure water that has been used by the patient. While it is not settled beyond dispute what particular bacteria cause each specific disease, it is an unquestioned fact that many diseases are produced by the self-multiplication of these microbes in the human system.

The conditions under which water containing organic impurities will develop disease-producing bacteria are unknown. Much depends on the condition of the system of the individual. One person may fall a victim to the disease, while others using the same water escape. People are slow to believe that there is danger from this source. A water may be bright and clear and give no evidence to the senses of smell, taste, and sight of the danger that lurks in it; or, the water may give evidence to the senses that it is full of impurities and yet may have been used for years in this condition without a single case of disease resulting. No one can say that disease will result from its use. The chemist can only say that the water is liable to develop disease-producing germs; the physician cannot say who will be the victim; the

disease may suddenly appear and work its havoc, often striking where least expected.

This possibility of disease and the unquestioned fact that typhoid fever has in so many cases followed the use of impure water, make it important that we be able to determine when a water contains considerable amounts of animal or vegetable matter.

Pure water does not occur in nature. The reason for this can be seen in the fact that water dissolves a very large number of substances. It is the most universal solvent known. Different substances, however, dissolve in widely different degrees. Six hundred volumes of ammonia and four hundred and fifty volumes of hydrochloric acid dissolve in one volume of water at ordinary temperatures. One volume of water dissolves one volume of carbon dioxide at the ordinary temperature and pressure. Under higher temperatures and pressures, — conditions found in the interior of the earth, — much larger quantities of carbon dioxide are dissolved. Such is the case with the waters of Selters and the Geyser Spring at Saratoga, which effervesce on exposure to the air. On the other hand, it requires 429 parts of water to dissolve one

part of calcium sulphate, $CaSO_4$, and 806,451 parts to dissolve one part of barium sulphate, $BaSO_4$. The solubility of some substances is greatly increased or diminished by the presence of others. Limestone, for instance, is practically insoluble in water unless the water already contains carbon dioxide. If carbon dioxide is present, it easily dissolves.

Even rain-water is not pure. The rain as it falls takes up particles of dust and the micro-organisms which are constantly present in the air, together with any gases that may be present. Large amounts of carbon dioxide, CO_2, are in this way absorbed and thus prepare the water to act on the limestone it comes in contact with in passing through the earth. Rain-water also absorbs nitrogen and oxygen from the air, and, during thunder-storms, the action of the electricity causes these gases to combine chemically with the water, thus forming nitrous acid, HNO_2, and nitric acid, HNO_3. In volcanic regions and near mills and cities water absorbs sulphur compounds, such as hydrogen sulphide, H_2S, sulphurous acid, H_2SO_3, and sulphuric acid, H_2SO_4. The air is thus purified by the first rain that falls, so that rain-

water, collected after it has been raining for some time and at a distance from the foul air of cities, is the purest water to be obtained in nature; but even this, when evaporated on a watch-glass or piece of clean platinum foil, leaves a slight stain, showing that it is not entirely free from foreign matter.

As soon as the rain comes in contact with the earth it becomes impregnated with solid substances varying with the character of the soil through which it flows. Streams flowing rapidly over sandstone rocks are less subject to contamination than any others both on account of the insolubility of the sandstone and because the flowing water is constantly exposed to the action of the oxygen of the air, nature's purifier, which unites chemically with the contained organic matter. On the other hand, the lakes and slow-flowing streams of alluvial regions contain large quanties of foreign substances, both inorganic and organic.

There is no question that the best water in nature for drinking and culinary purposes is that from deep-seated springs. This statement does not of course apply to spring water containing earthy matters in such quan-

tity as to warrant the name of mineral water. There is no doubt that some of these possess valuable remedial properties, but this very fact unfits them for use as a beverage. In other cases, so-called mineral waters are simply very pure forms of water, especially free from dissolved solid matter or containing minerals not harmful and even beneficial to the human system. Such waters are of course wholesome.

The water of wells may become polluted not only through the sources already mentioned, but in its passage through the earth may dissolve out numerous other substances both harmless and injurious. The worst contamination comes from the taking up of decaying matter, both that of vegetable and that of animal origin. The latter source of contamination is especially objectionable. In the case of shallow wells near human habitations the drainage often finds its way into the water with too insufficient filtration through the earth to remove the impurities. Flowing water when thus polluted purifies itself to some extent by oxidation. It is not well, however, to put too much dependence upon purification by this process. There is no

question that the water supplied to many towns from rivers is contaminated by the sewage of other towns located higher up on the rivers.

Unquestionably, wells are in most cases located too near sources of contamination. Just how near deposits of decomposing matter may exist without endangering the purity of the water cannot be stated. The character of the soil and the local conditions must be taken into consideration. There have been many undoubted instances where deposits at a distance of more than one hundred feet have caused decided pollution. Prof. W. R. Nichols quotes from a German authority a case which will serve as an illustration and as an example of the danger that exists in such deposits within the vicinity of a source of drinking water. The water of a well suddenly took on chalybeate properties, although it had never been known to possess such properties before. Upon looking for the reason it was found that a quantity of spoiled beer had been emptied at a distance of about one hundred and fifteen feet from the well. This acting as a reducing agent on the ferric oxide, Fe_2O_3, of the soil formed ferrous car-

bonate, $Fe\ CO_3$, which is soluble in water. It was evident that water from this spot had found its way into the well. Many other instances of a similar nature might be cited.

Channels are often opened by roots of trees through which polluted water may find direct entrance into a well. The water is sometimes brought considerable distances in this way without passing through sufficient soil to remove the contained impurities. Under such circumstances the danger of contamination is very great. Such roots should not be allowed to remain when they can possibly be removed. To avoid contamination from this and similar sources, it is advised that the sides of the well be cemented with a cement impervious to water, so that the water must pass down to the very bottom of the well before it can gain admission. This insures a greater amount of filtration through the soil and is an excellent arrangement in all cases.

TEST TO DETERMINE WHETHER WATER FROM A POSSIBLE SOURCE OF CONTAMINATION FINDS ITS WAY INTO A WELL.

It is an easy matter to determine whether water from a given spot finds entrance into a well. A determination of the chlorine present in the water is first made by the method to be hereafter described. Then a considerable quantity of common salt, NaCl, is deposited on the suspected spot and caused to dissolve. The chlorine in the water is determined daily for a number of days. If its amount is found suddenly to increase and after some days to diminish, it is evident that the dissolved salt has found its way into the well. It goes without saying that if the compounds of chlorine are washed in, other soluble impurities will also be carried in.

CHAPTER II.

ANALYSIS.

THE complete quantitative analysis of water is difficult. The processes are long and tedious, and require skilful and accurate work. An examination for sanitary purposes is comparatively easy and simple. To decide whether a water is suitable for drinking and culinary purposes, it is sufficient, in most cases, to examine it for free and albuminoid ammonia and for chlorine. If the results from these tests are good, there need be little hesitation in pronouncing the water wholesome. If these results are not good, the water may reasonably be considered doubtful. The apparatus and chemicals required for this examination are quite inexpensive. The processes may be very quickly learned, and are so simple that the results may be depended upon as accurate. To enable one to speak with entire confidence about a water, a most thorough examination is, of course, necessary. In certain cases an examination for poison-

ous metals must be made. It is desirable, of course, to know as many other facts as possible, but it is not essential for the simple purpose of saying whether or not the water contains substances injurious to health.

COLLECTION OF SAMPLES.

The water to be analyzed should be brought to the laboratory in a perfectly clean vessel. Nothing is better than a glass bottle with a glass stopper. Both the bottle and stopper should be carefully washed in the water that is to be analyzed before the bottle is filled. If the water is to be taken from a pump, or the faucet of a town-supply, allow a considerable quantity to run away before filling the bottle. If the sample is to be dipped from a well or spring, dip deep and avoid the surface water. The water should be analyzed as soon as possible. The micro-organisms increase in number with great rapidity in water that is allowed to stand, and, working on the contained impurities, materially change the character of the water. The containing vessel should be kept closely stoppered.

Color. — Pure water, in small masses, is very nearly transparent. In large masses, it has a delicate blue color. Freedom from other colors may be considered an indication of purity, but it is by no means true that a transparent water is necessarily free from contamination; many waters that are bright and clear show, on analysis, that much organic matter is present in solution. Nor, on the other hand, does it necessarily follow that a water is unwholesome and unfit for drinking purposes because it is tinted with other colors than blue. Water from peaty districts is often of a greenish or brownish color, and yet experience has shown that it is not unwholesome. Such water when first used may cause temporary derangement of the digestive organs, but after a time there is no further trouble. Such waters are in constant use in many places, and no permanent ill effects result from them. Notwithstanding the facts that neither the presence nor the absence of color is conclusive evidence of the fitness or unfitness of a water for drinking purposes, it is well to notice the color of the water under

examination. If strongly colored — especially if yellowish — the ammonia and chlorine tests should be applied with great care.

TEST FOR COLOR.

The color of the water may be determined with sufficient accuracy by looking down through the water in a tall glass vessel standing on a sheet of white paper.

If it is desired to be more particular, a long glass tube with glass ends may be filled with the water, and a white card arranged to reflect the light through the tube. A much greater depth of water can be examined by this method.

Turbidity. — Suspended particles of solid matter often give the appearance of color to water. If such water is allowed to stand until the suspended matter settles, or if it is passed through a filter, the foreign matter is removed, and the water is found to be very free from injurious substances. It is important to distinguish between turbidity and color.

TEST FOR TURBIDITY.

Look through a flask of the water toward the light. Suspended matter is easily seen,

especially if some dark object, as a bar of the window sash, is viewed through the water. If there is much suspended matter, filter before going on with the examination.

Taste.— Excess of impurities in water may sometimes be detected by the taste. A perceptible taste calls for a careful examination.

Smell.— Water giving off bad odors is not fit for drinking purposes. Such odors arise from the decomposition of organic matter, especially of animal matter. To detect the odors in less marked cases a large jar half filled with the water and closely stoppered may be set in a warm place for some hours. Take the odor immediately upon opening. If much decomposing organic matter is present hydrogen sulphide, H_2S, will be given off.

TEST FOR HYDROGEN SULPHIDE.

Place one quart of the water under examination in a stoppered bottle holding two quarts. Add two or three drops of sulphuric acid, H_2SO_4. Suspend above the water a strip of filter paper wet with lead acetate, $Pb(C_2H_3O_2)_2$. Stopper the bottle securely and put it in a warm place for some

hours. If hydrogen sulphide is present the paper will be blackened by the formation of lead sulphide, PbS.

Heisch's Test for Sewage Contamination.— A very good way to roughly decide whether or not water contains much matter of animal origin is to place some of it in a bottle and add a little sugar. Stopper the bottle tightly and set it in a warm place exposed to the light for some days. If the water remains clear it does not contain much organic matter, but a turbidity indicates contamination. This turbidity is due to the growth of a fungus which may sometimes be seen after the water has stood only a few hours if the bottle is held in front of a dark background and examined by reflected light. This growth is interesting under the microscope.

CHAPTER III.

EXAMINATION FOR TOTAL SOLIDS.

Apparatus needed: — A balance weighing to milligrams; a platinum evaporating dish holding somewhat more than 100 c. c.; a Bunsen burner or other means of heating.

The platinum evaporating dish is carefully cleaned and quite strongly heated. It is then cooled in contact with some good conducting surface. This is done that the dish may be in the same condition as nearly as possible at both weighings. Weigh the dish and place it on a water bath, which may be improvised from a beaker of suitable size to support the evaporating dish.

Place 100 c. c. of the water under examination in the dish and evaporate to dryness. The dish is allowed to remain on the water bath about ten minutes longer than is necessary to accomplish this to insure thorough drying of the residue. The dish is then cooled and weighed. From this weight sub-

tract the weight of the dish and, expressing the result in milligrams, we have the number of milligrams of solid residue in 100 c. c. of the water. Now 100 c. c. of water weigh 100,000 milligrams. Our result is, then, the number of milligrams of solid residue in 100,000 milligrams of water, or the number of parts of residue in 100,000 parts of water.

LOSS ON IGNITION.

Heat the residue in the platinum dish moderately and observe if it blackens. Then heat to low redness and observe again. Cool, weigh, and find how much the residue has lost in weight. If the residue blackens, particularly if it blackens in spots, organic substances are present.

The Loss on Ignition was formerly believed to indicate the amount of organic matter present, and this determination was considered important. It is now known that serious errors may result from the process, and very little importance is attached to the determination. The only important point is to notice if the residue blackens in spots.

A water should not be condemned on ac-

count of the solids contained, unless fifty or sixty parts in one hundred thousand are found. Even these limits may be exceeded. The local conditions must be considered. More will be said on this point later.

CHAPTER IV.

CHLORINE.

CHLORINE is very abundant in nature as one of the constituents of common salt, NaCl. On account of the abundance of salt in the soil and its ready solubility in water, it easily finds its way into the water of our wells and streams. Carried down by streams to the ocean, it is left there by the evaporation of the water, which returns to the earth to carry down another load of the salt. This accounts, or at least helps to account, for the saltness of the ocean. In the case of lakes which have no outlet, such as the Dead Sea and Great Salt Lake, the accumulation continues until the water becomes intensely salt.

Salt, as is well known, is very wholesome and its presence in a water should not in itself condemn the water. Wells situated near the sea, or where there are deposits of salt in the earth, often contain large amounts of chlorine and yet the water is perfectly whole-

some. It is because salt is one of the constituents of human waste that its determination in potable water becomes important.

Although chlorides are present in all soils and in all waters, the amount is usually small. When it is known that the wells of a given region contain a particular amount of chlorine, any great excess over this average amount in any particular well indicates contamination from human sources. Chlorides remain as evidences of past contamination after all other evidences have been obliterated, and resist in a marked degree the effects of filtration through the soil.

The value of a knowledge of the amount of chlorine present in a given water is only to enable us to compare it with other waters of the same region. In most places much more than one part in one hundred thousand renders the water open to suspicion and calls for a careful determination of the free and albuminoid ammonia. A water containing five or more parts in one hundred thousand may be condemned at once, unless the large amount can be accounted for by the proximity of the ocean or of salt deposits.

DETERMINATION OF CHLORINE.

Apparatus and Chemicals needed: — 25 c. c. burette; 100 c. c. graduate; solution of potassium chromate, K_2CrO_4, and a

STANDARD SOLUTION OF SILVER NITRATE.

Dissolve 4.79 grams of silver nitrate, $AgNO_3$ in one liter of distilled water. Each cubic centimeter of this solution contains silver enough to unite with one milligram of chlorine, forming silver chloride, AgCl, a white precipitate.

Preliminary Test, No. 1. — Acidify 100 c. c. of the water under examination by adding a drop or two of nitric acid, HNO_3. Then add two or three drops of silver nitrate. A white precipitate shows the presence of chlorine.

Preliminary Test, No. 2. — If test No. 1 gives no result, concentrate 100 c. c. of the water to 50 c. c. by boiling. Add a drop of nitric acid and two or three of silver nitrate. A white precipitate indicates chlorine.

These precipitates are soluble in ammonia, NH_4OH; insoluble in nitric acid, HNO_3. They blacken on exposure for some time to strong light.

QUANTITATIVE ESTIMATION OF CHLORINE.

To 100 c. c. of the water under examination add enough of the solution of potassium chromate to give it a distinct yellow tinge. Then run in from a burette the standard solution of silver nitrate. Stir, and repeat until the red precipitate remains permanent. The number of cubic centimeters of the solution of silver nitrate used equals the number of milligrams of chlorine in 100,000 milligrams of water, or the number of parts of chlorine in 100,000 parts of water.

This method of determining chlorine is based on the fact that chlorine has a stronger affinity for silver than has chromic acid. The red precipitate is silver chromate, Ag_2CrO_4. This cannot remain permanently until all the chlorine present has united with the silver, forming silver chloride, AgCl. Up to this point the chlorine displaces the chromic acid of the silver chromate which first forms. When all the chlorine has been thus combined, silver chromate is permanently formed. It is important to stir well, and to notice the first instant when the precipitate is permanent.

A substance used as the potassium chromate is in this reaction, to indicate when another reaction is complete, is called an *indicator*, and the point at which the red color becomes permanent is called an *end reaction*.

CHAPTER V.

FREE AMMONIA.

FREE ammonia is not tested for because it is injurious in itself in the amounts that are ever found in natural water. In these amounts ammonia is harmless. It is because ammonia is one of the first products of the decomposition of animal matter, and because its presence proves that impure matter has recently been present, and probably some is now present, that its determination becomes important.

Apparatus and Solutions Needed: — Distilling apparatus of glass; test tubes marked with a scratch of a file at 50 c. c. capacity; metric graduate; 25 c. c. burette.

NESSLER'S SOLUTION.

Dissolve 3.5 grams of potassium iodide, KI, and 1.3 grams of mercuric chloride, $HgCl_2$, in 80 c. c. of water. Add more solution of mercuric chloride until a slight pre-

cipitate of red mercuric iodide, HgI_2, remains after stirring. Now add 16 grams of potassium hydroxide, KOH, and water to make 100 c.c. The liquid should be of a light straw yellow. Keep closely stoppered in the dark. It may be sensitized at any time by adding mercuric chloride solution until the red precipitate is just perceptible.

STANDARD SOLUTION OF AMMONIUM CHLORIDE.

Dissolve 1.573 grams of ammonium chloride, NH_4Cl, in one liter of water free from ammonia. For use take 100 c.c. of this and add 900 c.c. of water free from ammonia. Each cubic centimeter of this solution contains .05 of a milligram of ammonia.

SOLUTION OF POTASSIUM PERMANGANATE AND POTASSIUM HYDROXIDE.

Dissolve 200 grams of potassium hydroxide, KOH, and 8 grams of potassium permanganate, $KMnO_4$, in 1,100 c.c. of water free from ammonia and boil down to 1,000 c.c.

WATER FREE FROM AMMONIA.

Acidulate with sulphuric acid, H_2SO_4, common distilled water and distil again. Reject the first part of the distillate, and if some ammonia remains treat the distillate with sulphuric acid and repeat the distillation. When a few drops of Nessler's solution produce no coloration, the distillate is known to be free from ammonia.

EXAMINATION FOR FREE AMMONIA.

Place 250 c.c. of the water to be examined in the flask of the distilling apparatus and add a pinch of sodium carbonate, Na_2CO_3, to render the water alkaline. Distil, receiving the distillate in the test tubes marked to hold 50 c.c. each. To the first 50 c.c. of the distillate add 2 c.c. of Nessler's solution; a brownish-yellow coloration will be produced.

The determination of the amount of ammonia is now made as follows: A second 50 c.c. test tube is taken and a measured quantity of the standard solution of ammonium chloride is run into it from a burette. The test tube is filled up to the 50 c.c. mark with

water free from ammonia and 2 c. c. of Nessler's solution are added. The brownish-yellow coloration produced is compared with that in the first 50 c. c. of the distillate. Allow five minutes for the color to develop. If the colors are not identical at the first trial the process is repeated with different measured quantities of the ammonium chloride until no difference of shade can be distinguished when the two test tubes are held side by side in different lights. It is well to look through the tubes towards the light and to view them by reflected light with both white and dark backgrounds; also to look down upon the surfaces when the tubes are held over both white and dark objects. This determination of the identity of the colors is the most delicate and most important step in the process. It will require some practice.

Continue the distillation until at least three tubes of 50 c. c. of the distillate are obtained. Nesslerize each in the manner described. Notice how much of the standard solution of ammonium chloride has been used for the three tubes. Each cubic centimeter of the solution of ammonium chloride contains .05 of a milligram of ammonia. Multiply .05 of

a milligram by the number of cubic centimeters used and we have the amount of ammonia in the 250 c.c. of water experimented upon. Divide by 2.5 and we have the number of milligrams of free ammonia in 100 c.c. or 100,000 milligrams of water. In other words, the number of parts of free ammonia in 100,000 parts of water.

The water remaining in the flask is to be used in the determination for albuminoid ammonia.

CHAPTER VI.

ALBUMINOID AMMONIA.

THE method of examination for albuminoid ammonia was devised by the English chemists Wanklyn, Chapman and Smith, and the principle of the method as stated by them "is the measurement of the nitrogenous organic matter in water by the quantities of ammonia yielded by the destruction of the organic matter." The term "albuminoid" is used because albumen is one of the substances which yield ammonia when treated by the process. Any organic substance yields it. The albuminoid ammonia is not something that already exists in the water, but is formed by the processes of the method from the contained organic substances. The amount of albuminoid ammonia found measures the amount of organic matter present in the water. Its determination, then, is of great importance.

EXAMINATION FOR ALBUMINOID AMMONIA.

The examination for albuminoid ammonia should be begun at the same time with the examination for free ammonia. Place 25 c. c. of the solution of potassium permanganate and potassium hydroxide in a flask with 200 c. c. of water free from ammonia. Boil and test the distillate for ammonia. Continue the distillation until there is no coloration when a little of Nessler's solution is added to the last few cubic centimeters of the distillate.

When the determination of the free ammonia has been completed, this solution of potassium permanganate and potassium hydroxide is added to the water remaining in the flask, and the distillation continued until three test tubes each containing 50 c. c. of distillate are obtained.

The amount of ammonia in each of these test tubes is obtained by comparison with the standard solution of ammonium chloride in the way already described for the determination of free ammonia. The three amounts added give the total amount of albuminoid ammonia in the 250 c. c. of water. The parts

per 100,000 are calculated as under free ammonia.

The following modifications of the processes of examination for free and albuminoid ammonia have been recommended by the Chemical Section of the American Association for the Advancement of Science. They may be used instead of the foregoing processes.

FREE AMMONIA.

"Two hundred c. c. of distilled water, together with 10 c. c. of the sodium carbonate solution (made by dissolving 50 grams of sodium carbonate, which has been strongly heated, in 250 c. c. of distilled water and the solution boiled down to 200 c. c.), are distilled down to about 100 c. c. in the retort in which the analysis is to be conducted, and the last portion of 50 c. c. nesslerized to assure freedom from ammonia. Then 500 c. c. of the water to be examined are added and the distillation carried on at such a rate that about 50 c. c. are collected in each succeeding ten minutes, and until a 50 c. c. measure of distillate is obtained containing only an inappreciable quantity of ammonia."

"Throw out the contents of the retort; rinse it thoroughly; put in 200 c. c. of distilled water, and 50 c. c. of the permanganate solution; distil down to about 100 c. c., and nesslerize the last portion of 50 c. c. to make sure of freedom from ammonia; add another portion of 500 c. c. of the water under examination, and proceed with the distillation and nesslerizing as with the first portion.

"The difference between the free ammonia of the first operation and the total ammonia of the second is to be taken as the albuminoid ammonia."

In both of these processes each 50 c. c. of the distillate is separately nesslerized, and the amounts added to give the results.

No hard and fast rules can be laid down in regard to the amount of ammonia which should condemn a water. It may be roughly stated that over .005 part of free ammonia and over .015 part of albuminoid ammonia render a water open to suspicion. More will be said on this point in another place.

CHAPTER VII.

NITRITES AND NITRATES.

NITROGENOUS organic matter, in contact with the air, develops micro-organisms which reduce the organic matter to nitrites and nitrates. These compounds also result, to a small extent, from the action of electricity in forming nitrous and nitric acids from the gases and water contained in the air, as previously noted. Nitrites and nitrates, in small quantities, are not in themselves injurious, but their presence, to any great amount, is believed to show that decomposing animal matter has recently been present. If the tests indicate that nitrites and nitrates are present in large quantities, the examination for albuminoid ammonia should be conducted with especial care.

TEST FOR NITRITES.

To 250 c. c. of the water under examination add two or three drops of acetic acid, $C_2H_4O_2$, and distil. The first portion of the

distillate is received in a test tube containing starch-paste and potassium iodide, KI, acidulated with pure sulphuric acid, H_2SO_4. If nitrites are present the paste solution will be turned blue, owing to the liberation of iodine and the subsequent formation of the iodide of starch.

To make the starch-paste, rub up a small quantity of starch with cold water, and then boil. A little of this is added to an aqueous solution of potassium iodide.

TEST FOR NITRATES.

Place a few cubic centimeters of the water under examination in a test tube, and pour upon it an equal volume of pure, strong sulphuric acid. When the mixture has become cool, add slowly and carefully, letting it run down the side of the tube, a solution of ferrous sulphate, $FeSO_4$. The formation of a dark ring at the boundary surface between the two liquids shows the presence of nitrates.

Water analysts differ very much in regard to the value of the determination of nitrites and nitrates. Some lay great stress on these results; others consider them of little practi-

cal importance. When the examination for albuminoid ammonia is carefully made, the above tests are sufficient for all practical purposes, as the results point to the same kind of contamination; that is, contamination from organic matter. If it is desired to make a more careful determination, the process recommended by the Chemical Section of the American Association for the Advancement of Science for determining nitrogen as nitrates and nitrites may be used. The nitrogenous compounds are decomposed by the galvanic action of the zinc-copper couple, and the ammonia formed is nesslerized in the manner already described. Use 4 c. c. of Nessler's solution each time.

The zinc-copper couple is made by immersing a piece of common sheet zinc in a solution of copper sulphate, $CuSO_4$ (5 grams in 300 c. c. of water free from ammonia). The zinc is first carefully cleaned in dilute hydrochloric acid, HCl, and rinsed free of acid. When the surface is well covered with the black deposit of copper, it is carefully washed by dipping in several changes of water free from ammonia.

Acidify 500 c. c. of the water under ex-

amination by adding pulverized oxalic acid, $H_2C_2O_4$. with constant stirring. Divide into two portions of 250 c.c. each in closely stoppered bottles. In one of these bottles place the zinc-copper couple and set the bottle in a warm place for twenty-four hours. The water in each is now nesslerized. No distillation is necessary. The water is decanted from the earthy oxalates which have settled to the bottom of both bottles. If the amount of ammonia in the water containing the zinc-copper couple is large it may be necessary to take only a few cubic centimeters at a time and dilute with water free from ammonia. The total amount of ammonia obtained from the water containing the zinc-copper couple minus the total amount from the other bottle is derived from the nitrogenous compounds and is denominated nitrogen as nitrates and nitrites.

CHAPTER VIII.

POISONOUS METALS.

Iron, Lead and Copper. — Place two porcelain evaporating dishes side by side in a good light. Into one pour 100 c. c. of the water under examination. Into the other, 100 c. c. of water known to be free from iron, lead and copper. Add a drop of ammonium sulphide to each and stir with glass rods. Compare the colors of the two waters. A dark coloration indicates the presence of one or more of the metals iron, lead and copper.

If a coloration is produced, add two or three drops of hydrochloric acid, HCl. A coloration due to iron sulphide disappears. That due to copper or lead sulphide remains. The presence of iron may be verified by adding a few drops of nitric acid, HNO_3, to another 100 c. c. of the water and boiling thoroughly. Then add two or three drops of potassium sulphocyanate, KSCN. If iron is present, red ferric sulphocyanate will be formed.

If a coloration remains after adding the hydrochloric acid, add about 1 c. c. of a strong solution of potassium cyanide, KCN. A color due to copper disappears. If a coloration still remains, lead is present. The presence of copper may be verified by adding several drops of a strong solution of potassium ferrocyanide, K_4FeCN_6. to a fresh portion of the water; a red-brown color will appear if copper is present. The presence of lead may be verified by Harvey's process.

Place two glass cylinders side by side. Into one pour 250 c. c. of the water under examination. Into the other, 250 c. c. of water known to be free from lead. Into each put .1 of a gram of pulverized potassium dichromate, $K_2Cr_2O_7$, and dissolve by shaking. If lead is present a turbidity due to lead chromate, rendered more visible by comparison with the second cylinder, will appear after a few minutes, and finally the lead chromate will settle to the bottom.

Lead and copper may also be distinguished by the following processes: Concentrate a considerable quantity of the water to a few cubic centimeters and pass hydrogen sulphide, H_2S, into it for some minutes. Filter and place

the precipitate in a porcelain crucible and heat very strongly. A drop or two of nitric acid is now added and carefully evaporated. The residue is dissolved in a few drops of water, and two or three drops of potassium iodide are added. If lead is present, lead iodide, PbI_2, recognized by its yellow color, is formed.

Evaporate to dryness a considerable quantity of the water. Dissolve the residue in a few drops of distilled water, add a drop or two of hydrochloric acid and pass hydrogen sulphide into it. Filter and place the precipitate in a platinum crucible with a drop of strong sulphuric acid. Heat strongly, and, if copper is present, copper sulphate, $CuSO_4$, will be formed. When cold, add a small quantity of distilled water and a few drops of ammonium hydroxide, NH_4OH. A beautiful blue color results. The presence of copper may be further confirmed by driving off the excess of ammonia and adding potassium ferrocyanide, K_4FeCN_6. The ferrocyanide of copper, a brown-red precipitate, is formed.

QUANTITATIVE EXAMINATION FOR IRON, LEAD AND COPPER.

Standard Solution of Iron.

Dissolve .496 of a gram of ferrous sulphate, $FeSO_4$, in one liter of distilled water. Each cubic centimeter contains .1 of a milligram of iron.

Standard Solution of Lead.

Dissolve .166 of a gram of crystallized lead acetate, $Pb(C_2H_3O_2)_2$, in one liter of distilled water. Each cubic centimeter contains .1 of a milligram of lead.

Standard Solution of Copper.

Dissolve .393 of a gram of crystallized copper sulphate, $CuSO_4$, in one liter of distilled water. Each cubic centimeter contains .1 of a milligram of copper.

Having determined that iron, lead, or copper is present, place the two porcelain dishes side by side again. Into one pour 100 c. c. of the water under examination; into the other, 100 c. c, of distilled water. Add a drop of ammonium sulphide to each and stir. Now slowly run from a burette into the dish

containing the distilled water the standard solution of the metal known to be present. Stir thoroughly and carefully observe when the colors in the two dishes are identical.

As each cubic centimeter of the standard solution contains .1 of a milligram of the metal, divide the number of cubic centimeters of the solution used by 10 and the result is the number of milligrams of the metal in 100 c. c. of the water, or the number of parts per 100,000.

A water may contain .15 to .20 parts of iron per 100,000 and not be condemned. Even .1 part per 100,000 of lead or copper renders the water dangerous. These amounts, which seem so minute, are sufficient to produce a very decided coloration in the 100 c. c. of water experimented upon.

Zinc. — Zinc may be looked for by Allen's test. Add ammonium hydroxide, NH_4OH, to 100 c. c. of the water under examination to slight alkaline reaction. Boil, filter if necessary, and add a few drops of potassium ferrocyanide, K_4FeCN_6. If zinc is present, even in very minute quantities, a white precipitate will be formed.

Reinsch's Test for Arsenic. — Add sodium

carbonate to one liter of the water under examination to slight alkaline reaction, and evaporate nearly to dryness in a porcelain evaporating dish. Place a small piece of clean, bright copper foil in a test tube with a little water, add a drop or two of hydrochloric acid and boil gently. The copper will remain bright if the reagents contain no arsenic. It is very important to determine this, as the reagents frequently contain a trace of arsenic derived sometimes from the glass of the bottles in which they are kept. Having determined that the reagents are free from arsenic, add the residue of the water under examination to the contents of the tube and boil again. If arsenic is present, a greyish deposit will be formed on the copper foil. To verify, dry the foil between pieces of blotting paper, make it into a compact roll, and place it in a glass tube of small bore closed at one end. The tube is easily prepared from soft glass tubing by heating the end until it closes, and blowing into it while hot to form a small bulb. Now heat the tube gently, and if the deposit on the foil is arsenic it will be driven off and deposited again as metallic arsenic on the cooler part of the tube.

CHAPTER IX.

HARDNESS.

WATER, especially when it already contains carbon dioxide, CO_2, easily dissolves out lime and magnesia from the rocks through which it flows. When impregnated with these substances, it is known as *hard water*. Such water is usually clear and brilliant. The hardness is most commonly due to calcium carbonate, $CaCO_3$. Water containing this salt in solution is thought to be beneficial in giving strength to the bones and in building up the muscular tissues of the body, and, unless the amount of mineral matter is large, is generally considered wholesome. Waters having in solution considerable amounts of calcium sulphate, $CaSO_4$, and of the salts of magnesium, are less wholesome, tending to cause dyspepsia and other derangements of the digestive organs.

When soap is added to a hard water, the water becomes turbid, and hard, gritty particles are formed. This solid, insoluble sub-

stance is *lime* or *magnesia soap*. Hard soap is a complex mixture of sodium oleate, palmitate, and stearate. The latter substance is most abundant, so that we may consider hard soap as essentially sodium stearate. When it is added to water containing calcium carbonate or calcium sulphate, the sodium and calcium exchange places and calcium stearate (lime soap) and sodium sulphate, or sodium carbonate, are formed. Similar reactions result with compounds of magnesium. Calcium stearate and magnesium stearate are insoluble in water; hence much soap is wasted in removing the calcium and magnesium before it can begin its proper cleansing action. When the hardness has been thus removed from the water the soap begins its proper action, but the detergent properties of the water are less on account of the presence of the gritty lime and magnesia soaps.

If water which is hard on account of the presence of calcium and magnesium carbonates be boiled, these salts are precipitated and form a crust on the interior of the vessel in which it is boiled, the well-known *fur* in our tea kettles. The water is thus rendered more soft. These deposits are

often the cause of much trouble in steam boilers. Soft waters are therefore preferred for this use. Hard water is also as a rule, less desirable than soft water for culinary purposes.

Hardness due to calcium and magnesium carbonates is called *temporary hardness*, because it is removed by boiling. That due to calcium and magnesium sulphates is not affected by boiling, and is called *permanent hardness*. Permanent hardness may be removed to some extent by the addition of ammonia, NH_4OH. This is often done in the laundry.

Apparatus and solutions:— 25 c. c. burette; 8 oz. bottle.

DR. CLARK'S SOAP SOLUTION.

Dilute 37 c. c. of ordinary 95% alcohol with 63 c. c. of distilled water and in this mixture dissolve 1 gram of best old castile soap. Each cubic centimeter should contain soap sufficient to unite chemically with one milligram of calcium carbonate, $CaCO_3$.

CHECK SOLUTION OF CALCIUM CHLORIDE.

As the amount of moisture contained in soap is a variable quantity, the preparation of Clark's Soap Solution by weighing the soap is not sufficiently delicate for accurate work. A check solution containing a known amount of calcium is needed. This may be prepared by dissolving 1.11 grams of fused calcium chloride in one liter of distilled water. Each cubic centimeter contains the same amount of calcium that one milligram of calcium carbonate does.

To test the soap solution, add 5 c. c. of the solution of calcium chloride to 70 c. c. of distilled water, This amount of water is used because distilled water uses up a certain amount of soap before a permanent lather is produced; and it has been found by experiment that 70 c. c. of distilled water require the same amount of soap that is required to unite with one milligram of calcium carbonate. If the soap solution is of the right strength, it will take just six cubic centimeters of it to produce a permanent lather on the water thus prepared. If the soap solution is not of the right strength, more

soap, or alcohol and water, should be added until the correct result is obtained. The soap solution deteriorates upon standing for a long time. It should be tested once in two or three months.

EXAMINATION FOR HARDNESS.

From a burette run Clark's soap solution in small quantities into 70 c. c. of the water under examination contained in the 8-ounce bottle. Shake thoroughly after each addition, and place the bottle, which should be stoppered, on its side. Repeat until the lather remains unbroken over the surface for five minutes. The number of cubic centimeters of the soap solution required to accomplish this gives the hardness of the water in degrees.

If we subtract one from this result, to allow for the 70 c. c. of water, we have the number of grains of the salts of calcium and magnesium contained in each imperial gallon. To convert grains per imperial gallon to parts per 100,000 divide by .7. There is no uniformity among chemists in expressing hardness. It is sometimes expressed in degrees,

sometimes in grains per imperial gallon, and sometimes in parts per 100,000.

The soap solution does not work well when more than 15 or 16 cubic centimeters of it are required for the 70 c. c. of water. In case of a very hard water, take 70 c. c. and dilute with 70 c. c. of distilled water, and proceed as before. A deduction of two must now be made to obtain grains per imperial gallon.

The above process determines the total hardness, or that due to the salts of lime and magnesium. If it is desired to distinguish the permanent from the temporary hardness, boil some of the water. Replace the water lost as steam by distilled water, and determine the hardness as before. The result is the permanent hardness. The difference between the total hardness and the permanent hardness is the temporary hardness.

TO DETERMINE IF BOTH LIME AND MAGNESIA ARE PRESENT.

Lime. — Add one gram of pulverized ammonium oxalate, $(NH_4)_2C_2O_4$, to a liter of the water, and shake. If lime is present, a white precipitate of calcium oxalate will be

formed. The amount may be determined by collecting the precipitate on a filter, drying and weighing.

Magnesia. — After filtering, add a little more ammonium oxalate, to be sure that all the lime has been removed. Then add some soap solution. If magnesia is present, magnesium soap (magnesium stearate), a white cloudiness or precipitate will be formed. From the weight of this precipitate the amount of magnesium may be calculated.

The amounts of calcium and magnesium salts may also be determined by first finding the total hardness. Then precipitate the lime by means of ammonium oxalate, and, after filtering out the precipitate, find the hardness of the filtrate. The last result gives the amount of magnesium salts present. Subtract this from the total hardness, and the result is the amount of lime salts present. The result may be expressed as grains per imperial gallon, or parts per 100,000.

CHAPTER X.

INTERPRETATION OF RESULTS.

No fixed rules can be laid down for the interpretation of the results of an analysis of water. The chief value of chemical analysis is to detect gross pollution. In such cases the results are decided and the conclusions to be drawn from them are plain. Large amounts of free and albuminoid ammonia in connection with much chlorine condemn a water at once. In other cases, it is very difficult to know what opinion to pronounce. Amounts of solids, chlorine, free and albuminoid ammonia, which would condemn water from some sources would be perfectly harmless in water from other sources. It is, therefore, very important to know the source of the water and the surrounding conditions. It is not to be inferred from these statements that the chemical examination of water is without value. Chemical analysis cannot decide everything, but it can furnish much valuable information, and is at present the

most reliable means of deciding upon the purity or impurity of water.

Absolutely pure water is less palatable and no more wholesome than water containing moderate amounts of mineral matter. Water from deep-seated springs or from very deep wells may contain comparatively large quantities of solids and still not be injurious. It depends upon what the solids are. If the amount contained is very large it becomes important to make a determination of the solids. Deep-seated water may also contain an excess of ammonia, which is derived from the decomposition of nitrites and nitrates which have been dissolved out of the rocks. This ammonia is not an indication of impurity. Chlorine may also be present in quantities that would condemn the water of a shallow well and yet the water may be good. The chlorine may be derived from deposits of salt or salt water which have existed in the rocks from the time of their formation. In the case of these deep wells the chief reliance is to be placed on the determination of the organic matter present, in other words, on the results of the albuminoid ammonia process.

In the case of shallow wells, the question is no less difficult. No one result is sufficient either to condemn or approve a water. If the water does not contain more than fifty or sixty parts of solids in 100,000 it may generally be considered wholesome, if the other indications are good. The amount of chlorine that may be present depends largely upon the local conditions. If near the sea or salt deposits in the earth, the amount may be large and not give reason in itself for condemning the water. The chlorine ought not, however, to exceed one part in 100,000, unless a good reason can be found. The principal value of a knowledge of the chlorine is to enable us to compare the water with others from similar sources. The wells of any region will show about the same amount of chlorine. If a particular well of this region shows a considerable excess over this amount, there is reason for suspicion. If the water of a river for a town supply shows much more than one part in 100,000, the reason should be carefully sought out. Good river water usually shows much less. Five or six parts of chlorine in the water either of a shallow well or of a stream show gross pollution.

In regard to the amounts of ammonia, Wanklyn, the originator of the albuminoid ammonia process, interpreted the results substantially as follows: a water which yields no albuminoid ammonia may be considered good, even though considerable free ammonia and chlorine are found. A water that yields less than .005 part of albuminoid ammonia in 100,000 may be regarded as very pure. A water is to be looked upon with suspicion if it yields .005 part of albuminoid ammonia and a large amount of free ammonia. If the free ammonia is very small in amount there may be as much as .010 part of albuminoid ammonia, but more than this is to be regarded as suspicious, and .015 part is sufficient to condemn the water.

Other well known chemists accept these interpretations and consider it established in ordinary cases that the total solids should not exceed fifty or sixty parts, the free ammonia, .005 part, the albuminoid ammonia, .015 part, and that the chlorine should not much exceed one part in 100,000. If any one of the results is in excess of these figures an intelligent judgment on the water can be reached only by a careful consideration of

all the results together, the location of the well and the surrounding conditions. It may be added that the presence or absence of nitrates will in these cases materially aid in forming an opinion.

It is to be remembered that a high degree of hardness makes a water less desirable, and that the permanent hardness or that due to the sulphates of lime and of magnesium is especially objectionable.

Iron should not be present above .2 part per 100,000, and very minute quantities of lead, copper, zinc, or arsenic condemn a water.

It cannot be denied that even with the most careful work in the chemical examination of water there remains an element of uncertainty, and that it is impossible in all cases to decide definitely whether or not danger is lurking in the water. On the other hand, it is just as certain that chemical analysis in many cases decides at once and beyond question that impurities are present, and in all cases affords much very important information. The results of analysis are most valuable when, by this means, the waters from a similar source are compared for a given

region. A series of such analyses establishes a standard of purity for the region, and any great departure from this standard indicates pollution.

CHAPTER XI.

MICROSCOPICAL EXAMINATION.

No examination of water for drinking purposes is complete without a microscopical examination. The number of forms of animal and vegetable life which may occur in water is almost unlimited, and anything like a complete enumeration and description of them would require volumes. Such a study of the microscopic forms must be left to the specialist. The microscopical examination of water for sanitary purposes does not call for the identification with long Latin names of all the forms present, nor is it necessary to make a quantitative determination, which, even by the most refined processes now known, is but a rough calculation. It is sufficient for ordinary cases and for the simple purpose of deciding in regard to the wholesomeness of the water, when the question is settled as to whether or not much life is present, and especially whether many different forms are contained in the water.

The presence of animal life is not necessarily to the disadvantage of a water supply. Ponds and streams abound in fish life and in certain forms of animalcules. There is no question but that fish and some of the animalcules aid in purifying the water by devouring matters which tend to pollute. It cannot be said of all the living organisms that they are injurious when taken into the human system alive. It is believed that many are not harmful when so taken. But after death their decomposing bodies may cause the development in the water of other forms which are injurious. A knowledge of the presence of these organisms is important as indicating that impurities of an organic character are present. Very few of the microscopic forms of life flourish in pure water. The decomposing organic matter of polluted water furnishes food for the development of the lower forms of life. A water containing these forms is objectionable, then, not simply from the fact of the presence of these disagreeble objects, but for the still more important reason that they prove the previous contamination of the water.

The microscopical examination naturally divides itself into two parts: First, the de-

termination of the presence of the grosser forms of life, and of débris resulting from life; second, the detection of bacterial forms.

LARGER FORMS OF LIFE.

The detection of the larger forms of life is a very simple matter and requires only a very slight acquaintance with the use of the microscope. Several methods of procedure have been in use.

A considerable quantity of water may be allowed to stand, protected from dust, in a tall vessel for twenty-four hours, and then all but a small remnant siphoned off by means of a piece of rubber tubing. The residue is to be examined.

Or, a piece of cotton cloth may be tied over a faucet or the nose of a pump to act as a strainer. It is removed after considerable water has passed through it and the collected material rinsed off in a beaker of water. After this has stood for some time all but a small residue may be siphoned off.

Better than either of these is the method proposed by Prof. Sedgwick of the Massachusetts Institute of Technology. A piece of fine wire cloth is made into a compact

roll and placed firmly in the lower end of the stem of a glass funnel. Above this a small portion of the stem is filled compactly with clean sand. Through this sand filter a considerable quantity of the water under examination is passed. The quantity used will vary with the purity of the water. Half a liter of very impure water is sufficient. Two or three liters or even more of purer water may be required for a searching examination. The wire cloth is now removed and the sand and collected matter are placed in a test tube with a very little water. The tube is gently shaken and allowed to stand a few seconds. The sand, because of its greater specific gravity, settles much more rapidly than the collected matter. When the sand has nearly all settled the water is decanted into another test tube. This water will contain nearly all of the collected matter. Some of this residue may now be placed by means of a pipette in a shallow cell on a glass slip.

The pipette may be made from a piece of soft glass tubing. A piece of the tubing about a foot long is held with its middle portion in the gas flame until the glass is softened. It is then pulled in the direction of

the length until it parts. The middle should be held in the flame during the whole operation. Break off the finely drawn out tips so as to leave small openings and two pipettes are ready for use. The pipette is used by lowering the small end into the water and when a sufficient amount has entered it the finger is placed over the open upper end and the pipette raised. The shallow cell is also very easy of construction. Rings of glass, gutta percha, or metal may be purchased for a trifle of any dealer in microscopical supplies. They may be cemented to the ordinary glass microscope slide, three inches long by one inch wide, with any good cement. Common white shellac dissolved in the smallest possible quantity of alcohol is good. Coat the under surface of the ring lightly with the cement and place it on the central part of the glass slide and set aside with a light weight on it until the cement hardens. When dry apply a second and perhaps a third coat of the cement around the outside of the cell.

The cell is filled even full and a thin glass cover of the kind ordinarily used in microscopic work is first moistened and then carefully slid over the cell from one edge. It is

important that the cell be completely filled and that the cover be used to avoid distortion of the objects due to the uneven surface of the water in an open cell. If the directions are followed minutely there will be no trouble and no disappointment. The cell is now ready to be viewed under the microscope. In this examination a low power of the microscope should be used. A general view may be taken with a two-inch objective, and afterwards a three-fourths or one-half inch objective may be used.

A closer examination may now be made. A very small drop of the residue may be placed on a microscope slide, and a cover glass laid over it. If too much of the water is used it will run out from under the cover glass. This should be avoided. Any overflow may be taken up with a piece of blotting paper. Higher powers of the microscope may be used in this examination. Use a three-fourths inch objective first, and follow it with a one-fifth inch. It is sometimes of advantage to use a stain to bring out the smaller and more transparent forms. A drop of one of the aniline stains, fuchsine, for instance, may be placed by means of a pipette

or glass rod at one edge of the cover glass, and a piece of blotting paper at the opposite edge. The stain will be drawn under by capillary attraction.

Besides the actually living forms, fibers of wool and cotton, grains of pollen, bits of vegetable tissue, grains of starch, scales of insects, diatoms, various silicious and carbonaceous mineral deposits and amorphous masses resulting from the decay of animal and vegetable life will, at times, be found.* It need not be said that the presence of such objects in considerable quantities condemns the water for drinking purposes.

In both of the processes but a small degree of manipulative skill is called for, and a person entirely unacquainted with the use of the microscope would soon be able to successfully and satisfactorily perform the operations. If much animal and vegetable life, or amorphous undissolved solid matter is present in the water, it cannot fail to be detected in this way.

*For the identification of the forms found the reader is referred to MacDonald's Microscopical Examination of Drinking Water.

CHAPTER XII.

BIOLOGICAL EXAMINATION.

Bacteria belong to the lowest order of plants. They are known in botany as schizomycetes, or fission-fungi. They are of various shapes, — spheres, short rods, longer rods, and filaments. They are abundant everywhere,— in the air we breathe, the water we drink, and the food we eat. They are the active cause of decomposition. Wherever decay is going on bacteria are present in abundance. Bread, boiled eggs, and various other articles of food exposed to a warm, moist air, soon develop variously colored spots. These are colonies of bacteria. If we touch one of these spots with a needle, and touch the needle to another piece of bread or egg exposed to moisture and warmth, similar spots appear after a day or two. Bacteria are causing the decomposition of the bread or egg, and this decomposition will go on until the whole mass is consumed. Given the

right conditions of temperature, moisture, and food, and the development of bacteria is enormously rapid. The number of varieties is also very large. As the name, "fission-fungi," implies, they multiply by fission. The rod-shaped forms divide transversely to the length; the spherical forms, in various ways. When the conditions are not right for propagation by this means many varieties form spores, which, even when the parent dies, continue alive awaiting the right conditions to develop and multiply without limit.

Many forms of bacteria are known to be harmless when taken into the human system. Other forms are known to produce disease when the condition of the system is right for their development. The infectious diseases are believed to be propagated by the communication and development of different forms of bacteria.

Even very pure water contains large numbers of bacteria. Two hundred germs per cubic centimeter is a low estimate for pure water. Water containing dead organic matter furnishes the right conditions for their development, and very impure water often contains several hundred thousand to the

cubic centimeter. As already stated, many forms are not injurious when taken with the water. Water which offers the right conditions for the development of bacteria is very likely to contain many different species, and among these different species there are likely to exist some of the pathogenic forms. The multiplication of the bacteria cannot, however, go on indefinitely unless the supply of organic matter is constantly replenished. This is the food on which they live, and if its supply is diminished the bacteria die of starvation. In the case of flowing water the oxygen of the air consumes the organic matter, and the bacteria perish. The stagnant water of swamps contains immense quantities of bacteria. It will thus be seen that the number of bacteria present is a sure index of purity or impurity, and the importance of the biological examination of water is manifest.

The examination for bacterial forms is much more difficult than the detection of the grosser forms of life, and calls for much greater skill and knowledge of the processes in the higher technique of microscopical manipulation. In the examination for bacteria the

higher powers of the microscope are needed. The large forms may be seen and even distinguished by a power as low as a good fifth-inch objective, but a tenth or a twelfth-inch immersion lens and a sub-stage condenser are needed for practical work that is to yield results upon which dependence may be placed.

It is to be observed also that the methods of biological investigations of water are still in their infancy and can hardly be said to have reached the stage of practical utility. Whatever may be developed in this direction in the future, it can only be said that there is not yet agreement among specialists as to results or as to interpretation of results. The field is still new and open and offers most fascinating ground for research. The advances in other lines of bacterial studies during the past few years, however, warrant the confident expectation that the time is not far distant when the biological examination of drinking water will be considered of much greater importance than the chemical examination.

Any extended study of bacteria requires cultures to be made by impregnating prop-

erly prepared sterilized media with the germs existing in the water and developing and isolating the different forms at suitable temperatures and under the most careful conditions. This is the work of the specialist. A detailed description of complete apparatus and processes would be out of place here, and the reader is referred to the standard works on bacteriology. Simple apparatus and simple processes will be described sufficient to give an idea of the methods used in these investigations.

Sterilizer. — In place of the complicated sterilizing apparatus sometimes employed by specialists, an ordinary kitchen steamer may be used and the work done over the kitchen stove.

A cheap form of sterilizer which has been placed upon the market within a few years for sterilizing milk, known as Arnold's Steam Sterilizer, serves the purpose excellently well. The source of heat may be a Bunsen burner or an ordinary oil stove. The water wastes but little, as the steam is condensed and returned to the vessel at the base. A hole may be cut in the cover through which a chemical thermometer mounted in a cork

may be inserted. When steady temperatures below 100° C are desired, the flame should be turned down, after the proper temperature has been reached, so as to just maintain that temperature for the required time. With a little practice this is very easy of accomplishment.

Gelatine medium. — For test tube and plate cultures the following medium may be employed. Soak fifty grams of the best gelatine in three hundred cubic centimeters of filtered distilled water over night, and then melt it over a water bath or in the sterilizer. Test it for acidity with litmus paper, and add sodium carbonate in small quantities and with constant stirring until it just faintly turns red litmus blue. Filter through a piece of muslin which has been heated for some time in the sterilizer. The filtering is best performed in the sterilizer, as the solution hardens too rapidly to filter at the ordinary temperature. The whole filtering apparatus may be set within the sterilizer, and sufficient heat maintained to keep the gelatine melted. If the filtered gelatine is not clear, add a little white of egg and boil again. To this is added

Liebig's Extract of Meat,	2 grams.
Water,	150 "
Peptone,	5 "

This gelatine medium remains solid only at comparatively low temperatures. If it tends to liquefy use a larger proportion of gelatine. It is an excellent culture medium during the colder part of the year, but will liquefy in the hotter seasons. If it is desired to make these cultivations during the summer months substitute five grams of agar-agar for the fifty grams of gelatine. Agar-agar is a gelatine made from certain seaweeds found in the Japan sea. It may be obtained of dealers in microscopical supplies. Soak it over night in salt water. Wash in distilled water and drain. Then put it into the 300 c. c. of distilled water and dissolve with heat. It will require a much longer time than the gelatine to dissolve. The rest of the process is as before.

The gelatine or agar-agar medium is now placed in a good sized flask having the mouth loosely plugged with cotton wool, and boiled. It may now be poured into sterilized test tubes, ten cubic centimeters to the tube. There should be placed in the mouth of each

tube a good sized plug of cotton wool which has been singed by passing it rapidly through the flame.

The test tubes so prepared may be set in beakers for supports and placed in the sterilizer. Here they are to be heated at least twenty minutes, or two successive days. They must now stand several days and be watched to see if they have been thoroughly sterilized. If this has been accomplished the gelatine medium will remain unchanged. If not, spots or turbidity resulting from the growth of bacteria will appear.

Tube Culture. — When thoroughly sterilized the gelatine medium is ready for use. The tubes may be used immediately when cold, or may be kept indefinitely ready for use. The most critical step of the operation is the introduction of the water under examination. Sterilize a pipette by heating it for some time in the sterilizer or by passing it rapidly through the gas flame. As soon as it is cool enough take up a small quantity of the water. Now gently remove the cotton wool plug of one of the test tubes with a twisting movement just enough to allow of the introduction of the pipette and deposit a

small drop of the water on the center of the upper surface of the gelatine and quickly replace the cotton wool plug. The tube is now to be set aside at the ordinary temperature and watched for the development of bacteria at the spot where the water touched the gelatine surface. It is sometimes of advantage to allow the gelatine to cool with the tube in an inclined position so as to present a larger surface to view. In inoculating with the water draw the pipette along the surface lengthwise of the tube so as to impregnate the gelatine medium in a narrow line. If the spot or line presents a changed appearance after a day, or after two or three days, the bacteria are growing.

Cover glass preparations. — Sterilize a cover glass by passing it rapidly through the flame several times. With a needle inserted in a handle and sterilized as above spread a very thin layer of the medium, now supposed to contain the bacteria, on the cover glass. With sterilized forceps pass the cover glass repeatedly through the flame until it is dry, taking care not to burn the deposit.

Now pour upon the cover glass a freshly prepared and filtered solution of methyl violet

or fuchsine in sufficient quantity to cover it. Allow it to remain twenty or thirty minutes; wash thoroughly with a gentle stream of freshly filtered distilled water. If there is much diffuse staining wash with alcohol to remove it. Dry again carefully over gentle heat. Now place a small drop of Canada balsam on the center of a sterilized microscope slide and lower the cover glass, prepared side down of course, upon the balsam and press upon it gently with a wooden toothpick or some similar instrument. It is now ready for examination under the microscope with a high power. The bacteria may be preserved indefinitely on the slide without further treatment.

Plate culture. — Have ready a test tube containing about five cubic centimeters of thoroughly sterilized gelatine medium and plugged with cotton wool. Melt the medium by placing the tube in a beaker of warm water. Into this a measured quantity of the water under examination is to be introduced. This may be very accurately done by dropping the water from a pipette, having first determined in the following manner how many drops the pipette delivers to the gram.

Place a watchglass on one pan of the balance and counterpoise. Add a gram weight to the pan containing the counterpoise. Now slowly and carefully drop water from the pipette into the watch glass until equilibrium is restored. Repeat several times to see if the pipette is constant in its action. If it is not, try others until one is obtained which is constant. With this pipette, sterilized of course, introduce a definite number of drops of the water under examination into the test tube of melted gelatine medium. If the water is known to be quite impure two or three drops will be sufficient. With quite pure water a whole gram may be used. This water is to be thoroughly incorporated with the gelatine medium by repeated shakings while the medium is melted. Care must be taken not to heat the medium more than is necessary to keep it melted, as otherwise the germs in the water might be killed. During the whole process the test tube is to be kept plugged with the cotton wool, the plug being loosened just enough to allow the introduction of the pipette and immediately firmly replaced.

Now sterilize a plate of common window

glass four inches square by passing it rapidly through the gas flame. The greatest care must be taken during the remaining operations to avoid contamination from the floating germs of the air. The work should be done in a room as free from dust as possible and with the windows and doors closed to avoid currents of air. The glass plate is held with the side upon which the flame has played down until it is cool enough to be placed horizontally on a mixture of ice and salt with the sterilized side up. The contents of the test tube are now quickly poured upon the plate and a bell glass inverted over it. Immediately replace the cotton plug and rotate the test tube in cold water until the small amount of gelatine medium remaining in it has hardened. The bell glass may be a common tumbler, though one of larger diameter is desirable. The bell-glass should be sterilized by washing it with a weak solution of mercuric chloride, $HgCl_2$, made by dissolving one gram of mercuric chloride in one liter of filtered and boiled distilled water. The edge of the bell glass should be smeared with vaseline to make it fit the plate air-tight. The plate is kept in a horizontal position

over the ice mixture until the gelatine hardens. It is then set away to be watched for several days.

Enumeration.—If the water was thoroughly incorporated with the gelatine medium each germ that it contained will be the center of a growth. The colonies can be seen with the naked eye, or the bell glass may be removed and the plate placed under the microscope with a low power. The number of colonies may be counted. Inspect the test tube, and if any colonies have developed in the small remnant of gelatine medium left in it, add them to the number counted on the plate. The whole number of colonies found represents the number of bacteria in the fractional part of a cubic centimeter of water used. It is now an easy matter to calculate the number of organisms per cubic centimeter, which is the usual form of expression. If the number of colonies on the gelatine plate is very large the counting becomes tedious and difficult. The calculation may be much abridged by having another glass plate, ruled in squares. The ruling may be done with a glazier's diamond, or a plate may be extemporized by ruling it

with pen and ink. This plate is placed above the gelatine plate, with a thin sliver of wood at each corner to prevent it from coming in contact with the gelatine. The whole may be placed under the microscope with a low power, and the colonies in a number of squares counted. Find the average number in a square, and multiply this result by the number of squares covered by the gelatine medium, and calculate the number per cubic centimeter of water.

The bacteria may be examined under a high power by making a cover glass preparation as previously described, or a cover glass may simply be laid over a colony, and the plate placed on the microscope stage when it is ready for examination under an immersion lens.

Potato Culture. — Sterilize a plate of glass four inches square, and a bell glass, by washing in a weak solution of mercuric chloride. Place a piece of filter paper, sterilized in the same way, on the glass plate. In the top of the bell glass put a piece of filter paper wet with filtered and boiled distilled water, and invert the bell glass over the plate. Boil a potato. With a knife, sterilized by passing

it several times through a gas flame, peel the potato, and cut a slice about half an inch thick from the middle portion. Place this on the sterilized filter paper on the glass plate. With a sterilized pipette draw a line of the water under examination across the potato, and quickly re-cover with the bell glass, smearing the edge with vaseline. All of these operations must be performed with as little exposure to the air as possible. The apparatus may now be set aside, to be watched for several successive days. The growth of bacteria is easily detected by the color, or by a peculiar pellucid appearance of the potato along the line of growth. Examine by means of cover glass preparations. This method has been much used in cultivation of the typhoid bacillus.

Rindfleisch's Method for Typhoid Bacillus. — A drop of the suspected water is placed on a sterilized cover glass, dried, stained by allowing it to stand half an hour covered with a solution of methyl violet, washed in filtered distilled water, dried, and mounted in Canada balsam. Rindfleisch found deeply blue colored rods about .0002 mm. thick, and forming filaments up to .05 mm. long. It is characteristic of this bacillus, when cultivated

on potato, that it can be recognized by the naked eye, even after three or four days, only by a peculiar moist appearance of the potato.

Bacilli similar to these have been found in the mesenteric glands and the spleen of persons who have died of typhoid fever, and in the dejecta of typhoid patients. It has also been found that this bacillus thrives in milk as well as on potatoes. Injections of this bacillus into rabbits and monkeys have caused a disease similar to typhoid fever in man, and when the spleen and mesenteric glands of these animals were examined these bacilli were found in them. Notwithstanding these facts, there is a dispute among the highest authorities as to whether typhoid fever is caused by these bacilli, or by another kind of bacteria, — a form of micrococcus, minute spherical organisms. These micrococci are always found in large numbers in the bowels of persons who have died of typhoid fever. It has been suggested that typhoid fever is due to the combined action of the micrococci and the bacilli, the first preparing the bowels and the system generally for the action of the bacilli.

In all culture experiments the greatest care must be taken to avoid contamination from

the germs existing in the air. All apparatus used should be carefully sterilized by heating in the sterilizer, in the naked flame, or by washing in a solution of mercuric chloride. Too great stress cannot be laid on thoroughness in these particulars. When it is necessary to expose the culture medium to the air for the introduction of the water under examination, the exposure should be the briefest possible and under the most guarded conditions. The cotton wool plug should not be wholly removed unless it is absolutely necessary. Pipettes should be introduced by pushing them through between the plug slightly loosened and the side of the tube, and the plug immediately firmly replaced. The exposure should not be made in currents of air. A number of cultivations should be made for each sample of water examined and the results compared. In making cover glass preparations care must be taken that bacteria are not introduced during the process. Only freshly filtered distilled water should be used to wash the cover glass. The staining fluid should be freshly filtered. A blank experiment may be made to see that the materials used are free from bacteria.

Other stains than those already mentioned may be used. Almost any of the aniline dyes soluble in both alcohol and water are good. Contrast stains are often employed. After the cover glass preparation has been stained in the manner described, it is washed in alcohol until the greater part of the stain has been abstracted from the ground tissue and then submitted to the action of some other stain. Bismarck-brown and fuchsine, vesuvin and methyl-violet, vesuvin and methyl-blue are good contrast stains. They are used in alcoholic solution by preference.

It is to be remembered that bacteria are exceedingly minute. The apparent size under the microscope will, of course, depend upon the powers used. The first attempt to distinguish them often ends with disappointment, especially when too low a power is employed. The filamentous forms are readily recognized, but under a fifth-inch objective the largest individual forms are but mere specks. But even with this power a little patience and careful search will bring into clear view the short, straight rods and minute spheres of successful cultures,